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UBC.P-005-2
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Withers et al.

Serial No.: 09/837,711 Examiner:E. Slobodyansky

Filed : April 17, 2001 Art Unit:1652

For : Methods and Compositions for the Synthesis of Oligosaccharides Using Mutant Glycosidase Enzymes

RESPONSE TO OFFICIAL ACTION

Asst. Commissioner for Patents
Washington, D.C. 20231

Sir:

This is in response to the Official Action mailed November 21, 2001 for the above-captioned application. Reconsideration of the application in view of the remarks herein is respectfully requested.

Applicants request an extension of time sufficient to make this paper timely, and enclose the appropriate fee. The Commissioner is authorized to charge any additional fees or credit any overpayment to Deposit Account No. 15-0610.

Applicants confirm the election of Group I (claims 40-55) and the election of β-galactosidase as the enzyme species. Claims 40-50 and 55 are generic with respect to the elected species, and these are the claims which the Examiner has considered in the Office Action.

Applicants enclose a Form PTO-1449 listing the references of record in the parent case, and request that these references be made of record. Copies of the references should be

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I hereby certify that this paper and any attachments named herein are being deposited with the US Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231 on May 21, 2002.

Marina T. Larson

Marina T. Larson, PTO Reg. No. 32,038

May 21, 2002
Date of Signature

available from the parent cases, Serial No. 08/571,175, now US Patent No. 5,716,812 and/or 09/091,272, now US Patent No. 6,284,494 . If a fee is due with this submission, the Commissioner is authorized to charge the fee to Deposit Account No. 15-0610.

The Examiner rejected claims 40-50 and 55 under 35 USC § 112, first paragraph, as lacking a written description. Applicants respectfully traverse this rejection. To the extent that the written description requirement is separate from the enablement requirement, it imposes a requirement that the specification show that the Applicant really had possession of the invention as claimed as of the filing date of the application. This inquiry is made based on the words in the specification. It does not depend on unpredictabilty in the art, but rather on whether there is a clear expression that establishes that the inventors recognized that which is claimed as their invention. In the present case, there can be no issue because the language of the specification directly parallels that which is in the claims.

First of all, the title bespeaks the generality of the inventive concept, i.e., the use of "mutant glycosidases" generally. In the Summary of the Invention section (Page 4) the specification refers to "mutant glycosidases". On page 7, the specification states that "the present invention provides mutant forms of both retaining and inverting enzymes" and lists many enzymes from which mutant glycosidases can be prepared using the methods disclosed in the specification. Furthermore, claim 18 of the PCT application was plainly a generic claim encompassing the class of mutant glycosidases generally. Where the claim at issue is substantially the same as a claim presented in the original application, there is a strong presumption that written description support exists. The Examiner has neither addressed nor overcome this presumption.

For the foregoing reasons, Applicants respectfully submit that the claims meet the requirements of the written description requirement of 35 USC § 112, first paragraph, and that the Examiner has failed to present a complete rejection under this section. Withdrawal of the rejection is therefore urged.

The Examiner also rejected claims 40-50 and 55 under the enablement portion of 35 USC § 112, first paragraph. This argument is predicated on an assertion that the art is

unpredictable, and that the disclosed methods may not work in other enzymes. Applicants respectfully traverse this rejection.

As held in *In re Bowen*, 492 F. 2d 859, 181 U.S.P.Q. 48 (C.C.P.A. 1974), where the operability of materials beyond the scope of specific examples is challenged by the Examiner the burden is on the Examiner to present reasoned scientific argument as to why the stated method would not be expected to work. It is not sufficient to simply state that the claims are broad and the examples few. The Examiner must explain why a person skilled in the art would doubt the objective truth of an applicants statement that the invention has applicability and operability beyond the specific disclosed examples.

The Examiner states that "since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes in a protein's amino acid sequence would result in the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence are responsible for the requisite mechanism of action, subtrate, stereo- and regio-specificity, and detailed knowledge of the ways in which the proteins' structure relates to its function." This is a very generalized statement, which fails to take into account the scope of the claims or the disclosure. The claims are not broad enough to encompass any enzyme, or any protein. They only encompass glycosidase enzymes that, in the wild-type enzyme have "two catalytically active amino acids with carboxylic acid side chains within the active site." Further, the claims are limited to those enzymes with a mutation at one of these two catalytically active amino acids with an amino acid having a smaller, non-carboxylic acid side chain. The specification provides a mechanistic explanation of how the enzymatic reaction occurs, and why the claimed amino acid change results in the change of reaction type. The Examiner has not provided any reasons why a person skilled in the art would doubt that enzymes mutated at one of the two specified amino acids would have glycosynthase function,[1] and thus has failed to meet the burden of establishing a prima facie case for lack of enablement.

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[1] The term "glycosynthase" has been adopted in the art to describe a mutant enzyme which has lost its normal glycolytic glycosidase function, while retaining the ability to catalyze a coupling reaction.

Applicants further point out that the literature published subsequent to the effective filing date of this application reports several additional glycosynthases. Nashiru et al., Angew Chem. Int. Ed. 40: 417-420 (2001) (copy attached as Exhibit A), disclose a glycosynthase derived from a mannosidase (one of the types of glycosidases listed on page 7 of the application). in which the active site nucleophile Glu519 was converted to alanine or serine. Both provided mannosynthase activity, although the Glu519Ser mutant was more active. Trincone et al., Bioorganic & Medicinal Chem Lett 10: 365-368 (2000)(Exhibit B), disclose a mutant glycosynthase derived from the β-glycosidase of *Sulfolobus solfataricus*. These mutant forms change the active site glutamic acid residue (Glu387) to either alanine or glycine. Mayer et al., FEBS Letts 466: 40-44 (2000)(Exhibit C) describes a Glu358Ser mutant of *Agrobacterium* β-glycosidase (a different mutant of the enzyme which is used in the specific examples of the application) which has glycosynthase activity. Mayer et al., Chem & Biol 8: 437-443 (2001)(Exhibit D) describes mutants Glu358Cys and Glu358Gly of *Agrobacterium* β-glycosidase and shows them to have glycosynthase activity. Fort et al., J. Amer. Chem. Soc. 122: 5429-5437 (2000)(Exhibit E) describes a glycosynthase prepared by replacing the catalytic nucleophile Glu197 in endocellulase Cel7B from *Humicola insolens* with alanine. Malet et al., FEBS Lett. 440: 208-212 (1998) describe mutant forms of glucanases from *Bacillus licheniformis*. The paper notes that two glutamic acid residues Glu138 and Glu134 have been identified as the catalytic acid/base, and the nucleophile respectively. The mutant form Glu134Ala was prepared and shown to act as a glycosynthase.

In addition, Applicants submit herewith a declaration which describes a glycosynthase derived from *E. coli* LacZ β-galactosidase. Wild-type LacZ has within the active site a glutamic acid residue as amino acid 537 which is catalytically active. In the mutant form described in the declaration, this glutamic acid is replaced with a smaller amino acid with a non-carboxylic acid side chain, specifically serine. Coupling of β-D-glucopyranosides and an α-D-

galactosyl fluoride, or a β-D-cellobioside and an α-D-galactosyl fluoride are demonstrated. Thus, it is clear that the modifications to the active site of LacZ β-galactosidase as described in the present specification result in an enzyme which can be used in the method of claim 40.

In view of the lack of a *prima facie* case supporting the lack of enablement rejection, and the additional evidence filed herewith, Applicants submit that the rejection under 35 USC § 112, first paragraph should be withdrawn.

The Examiner rejected claims 40-50 and 55 for obviousness-type double patenting in light of claims 1-17 of US Patent No. 5,716,812 and claims 1 and 2 of US Patent No. 6,284,494. Applicants will submit an appropriate terminal disclaimer upon receiving an indication that the application is otherwise in form for allowance.

Respectfully submitted,

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